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Supercritical CO_2 extraction of resveratrol and its glycoside piceid from Chinese traditional medicinal herb *Polygonum cuspidatum*

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Abstract: Resveratrol (3,5,4′-trihydroxystilbene) and its glycoside piceid are phenolic compounds found to be beneficial to health. In this paper, resveratrol and piceid were successfully extracted from the Chinese traditional medicinal herb *Polygonum cuspidatum* (Hu Zhang in Chinese), with supercritical CO_2 plus ethanol modifier. The effects of ethanol content, pressure and temperature on the yields of resveratrol and piceid were investigated. Results showed that the extraction pressure was the main factor for the effective extraction of the two substances in the presence of ethanol modifier, especially for piceid. The optimal extraction condition was obtained: 30 MPa, 50 °C, (100 ml l^{-1}), ethanol and 20 kg h^{-1} of CO_2, at which the yields of resveratrol and piceid were 7.1 g kg^{-1} and 16.0 g kg^{-1}, respectively. The yields obtained by supercritical fluid extraction (SFE) was comparable with those obtained by traditional organic solvent methods, indicating that SFE is an alternative method for extraction of the two compounds.

Keywords: resveratrol; piceid; *Polygonum cuspidatum*; supercritical CO_2; extraction

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) and its glycoside piceid (Fig 1) are natural phenolic compounds of plant origin, which have demonstrated a spectrum of health-promoting roles such as protection against oxidation, heart diseases and cancers,[1–5] and are now being used as the main bioactive components of some medicines and functional foods in China. The two compounds usually occur together in red wines, grapes, peanut, etc and especially in grapes.[6] Recent studies[7] show that they are also abundant in *Polygonum cuspidatum*, a Chinese medicinal herb that is generally called Hu Zhang, Zi Jinlong or Banzhang and is widely distributed in China. The root of this plant has long been used for treatment of scalds and burns.[8–10] Because there have been reports that *P cuspidatum* contains much more resveratrol and piceid than grapes, very recently *P cuspidatum* is being paid increasing attention as a new resource for resveratrol and piceid. Chen *et al*[11] tried to extract resveratrol from *P cuspidatum* with organic solvent, and they[12] also purified the two compounds from the plant by high-speed counter-current chromatography.

Supercritical fluid extraction (SFE), which uses primarily CO_2 as extraction medium, has been widely used for the extraction of nonpolar substances such as oils from natural plants. Recent literature shows that it can also be used for extraction of polar compounds, such as flavonoids and polyphenolics, by adding a proportion of polar solvent, such as methanol and ethanol, as modifier.[13–16] However, to our knowledge, there are no reports on SFE of resveratrol and piceid from *Polygonum cuspidatum*.

The purpose of this paper is to explore the feasibility of extraction of resveratrol and piceid from *P cuspidatum* by SFE.

MATERIALS AND METHODS

Materials and reagents

Commercial roots of *Polygonum cuspidatum* were purchased from Shanghai Huifeng medicine shop (Shanghai, China), which were first pulverized into 60 ~ 80 meshes and then dried in a ventilative oven at 60 °C to obtain the products with 50–100 g kg^{-1} water before extraction. All-*trans*-resveratrol and all-*trans*-piceid standard samples (Purity >950 g kg^{-1}) were from Aldrich Chemical Co (Milwaukee, WI, USA). Carbon dioxide (999.9 g kg^{-1}) was provided by Minhang Gas Supply Station (Shanghai, China). Ethanol (AR grade, 95%) was purchased from Shanghai Chemical Reagent Shop (Shanghai, China).

Supercritical CO_2 extraction (SFE)

The pulverized dried roots of *P cuspidatum* were extracted with HA121-50-01 SFE equipment (Haian

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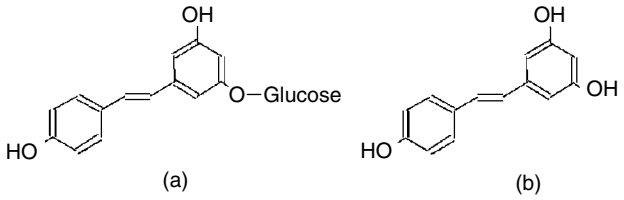

Figure 1. Molecular structures of (a) piceid and (b) resveratrol.

Supercritical Fluid Extraction Ltd, Nan Tong, China) consisting of dual pumps for addition of carbon dioxide and modifier. The extraction process can briefly be described as follows: One hundred grams of pulverized roots of *P cuspidatum* was packed into a 1-l sample cartridge inside an extractor which was preset at a extraction pressure and temperature. The CO_2 from cylinder was firstly liquefied and then pumped into a mixing vessel, where it was premixed with a certain proportion of ethanol pumped from modifier vessel, and then was pumped into the extractor to extract resveratrol and piceid. The extracted products and CO_2 were separated in a separator (5 MPa, 40 °C), where the extracts were precipitated at the bottom of the separator and the CO_2 gas went back from the top of the separator for the next extraction cycle. Three replications were carried out for each specified extraction condition. Each extraction experiment lasted for 2 h. Table 1 shows the specified extraction conditions, the average weights of dry extracts and resveratrol and piceid concentration in the dry extracts obtained from 100 g of roots of *P cuspidatum* at different conditions.

HPLC Chromatography

The extracted products (50 mg) were dissolved in ethanol to form a 100 ml solution, which was filtered through a 0.45-μm membrane and then assessed on a Waters 2690 HPLC instrument using a 5 μm particle size Hypersil Si column (4.6 mm × 150 mm) (Chrompack, Bergen op Zoom, The Netherlands) at 30 °C. The flow phase was a mixture of methanol and water (40:60, v/v) and the flow rate was 1 ml min^{-1}. The injection volume was 10 μl. The elution peaks were detected at 303 nm. The resveratrol and piceid contents in extracts were determined by comparison of their peak area with that of standard samples in HPLC chromatogram. The concentrations of standard solutions ranged from 0.1 to 100 μg ml^{-1}.

RESULTS AND DISCUSSION

SFE extraction

In our preliminary experiments, it was found that resveratrol and piceid could not be extracted out without modifier. Therefore the modifier ethanol was used to promote extraction. Figure 2(a) shows the effect of ethanol content on the yields of resveratrol and piceid (the yields were defined as the weights of resveratrol and piceid obtained from the each kilogram of dried roots of *P cuspidatum*) keeping conditions at 30 MPa, 50 °C and 20 kg h^{-1} flow rate of CO_2(vCO_2). As can be seen, the yields of resveratrol and piceid increased with the increase of ethanol content and reached the highest value at 100 ml l^{-1} ethanol and then tended to a level out later on. So 100 ml l^{-1} ethanol was required for the effective extraction of the two compounds. Our result was consistent with the previous reports[6,13–16] that polar compounds, especially phenolic compounds, could be extracted by applications of supercritical CO_2 with ethanol as modifier. A mechanism by which the polar compounds resveratrol and piceid could be extracted by supercritical CO_2 plus ethanol therein could be as follows. The molecular structures of resveratrol and its glycoside piceid contain hydroxyl groups, which could form hydrogen bonds with the hydroxyls of ethanol

Table 1. The extraction design, the average weights of dry extracts and resveratrol and piceid concentration in the dry extracts obtained from 100 g of roots of *Polygonum cuspidatum* at different conditions

	Technological parameters				Extracts		
Number	P (MPa)	*T* (°C)	vCO_2 (kg h^{-1})	Ethanol (ml l^{-1})	Dry weights (g)	Resveratrol[a] (g kg^{-1})	Piceid[b] (g kg^{-1})
1	30	50	20	4	6.1	66.1	115.2
2	30	50	20	7	9.4	64.2	117.1
3	30	50	20	10	12.4	57.4	129.3
4	30	50	20	14	12.5	57.3	129.4
5	30	50	20	17	12.5	51.7	130.1
6	10	50	20	10	5.1	39.6	20.5
7	15	50	20	10	8.2	72.1	24.6
8	20	50	20	10	9.6	68.1	94.1
9	25	50	20	10	12.1	58.4	132.2
10	35	50	20	10	12.5	56.7	125.4
11	30	35	20	10	11.9	51.9	109.3
12	30	45	20	10	12.0	55.4	125.1
13	30	60	20	10	11.8	55.2	119.4

[a] Resveratrol content in dry extracts.
[b] Piceid content in dry extracts.

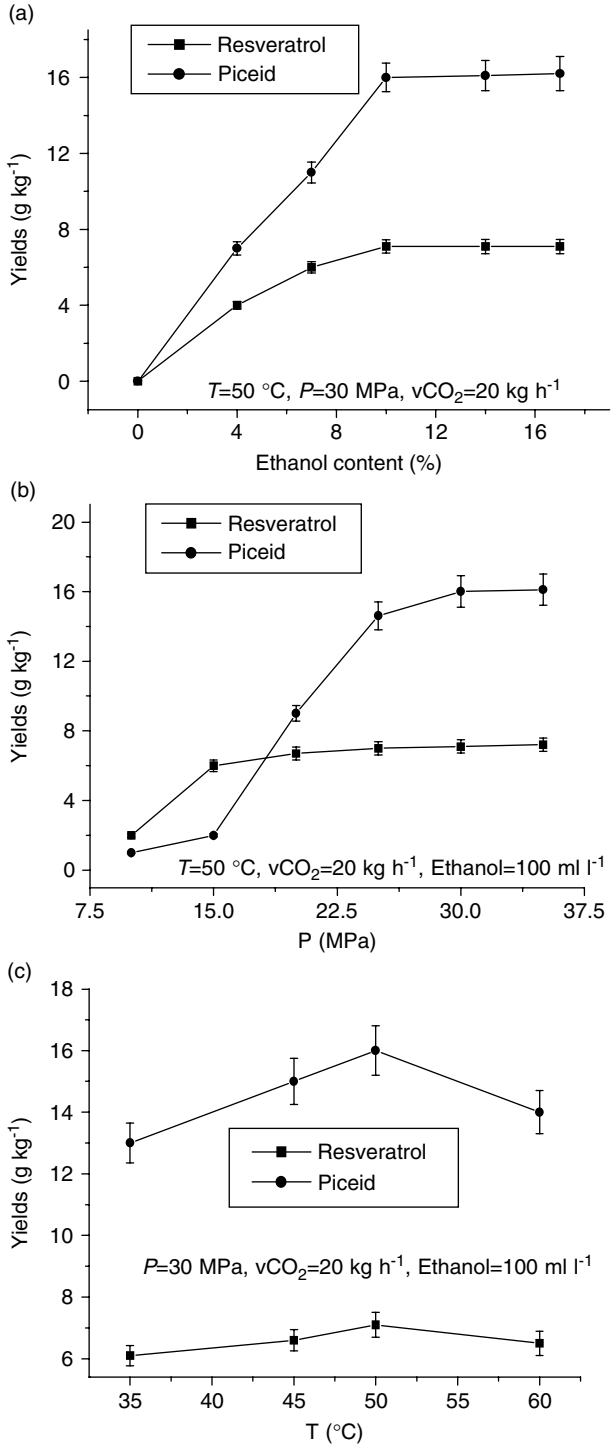

Figure 2. Effect of (a) ethanol content, (b) pressure, and (c) temperature on the yields of resveratrol and piceid.

molecules, and the interaction of hydrogen bonding enables resveratrol and piceid to be extracted by SFE. Moreover, it can be seen that the 100 ml l^{-1} ethanol was sufficient to form the hydrogen bonding to the maximum extent.

Figure 2(b) shows the effect of extraction pressure on the yields of resveratrol and piceid keeping conditions at 100 ml l^{-1} of ethanol, 50 °C and 20 kg h^{-1} of CO_2. The yield of resveratrol increased with increase in pressure and levelled out after 15 MPa; in contrast, the yield of piceid was very low before 15 MPa, increased significantly after 15 MPa, and levelled out after 25 MPa. Pascual *et al*[6] have successfully extracted resveratrol from grapes using supercritical CO_2 at 15 MPa. On this point, our result agreed with theirs. However, we, for the first time, report that only when pressure was increased to above 25 MPa could piceid be extracted effectively from *P cuspidatum*. Piceid, the glycoside of resveratrol, has a stronger polarity than the parent resveratrol, so its effective extraction needed higher solvent power the mixture of CO_2 and ethanol. Under the above from conditions, increasing pressure could lead to enhancement of the density of CO_2, and therefore enhancement of the solvent power of the mixture to extract piceid.

Figure 2(c) shows the effect of extraction temperature on the yields of resveratrol and piceid keeping conditions at 100 ml l^{-1} of ethanol, 30 MPa and 20 kg h^{-1} of CO_2. An extraction temperature in the range 35–60 °C affected the yields of extracted products slightly. The most suitable extraction temperature was 50 °C; the yields of resveratrol and piceid decreased slightly at temperature lower or higher than 50 °C. It is known that increasing temperature has a twofold, effect on extraction: on one hand, increasing temperature could lead to the increase of mass transfer, which is beneficial to extraction; on the other hand, it could lead to the decrease of density of CO_2, which is harmful to extraction. In our work, when temperature was increased above 50 °C, the harmful effect of the increasing temperature predominated, leading to the loss of yields of extracted products. Therefore, 50 °C was chosen as the best temperature.

From the above discussion, the optimal condition for extraction of resveratrol and piceid are as follows: 100 ml l^{-1} of ethanol, 30 MPa, 50 °C and 20 kg h^{-1} of CO_2.

HPLC chromatogram

The HPLC chromatogram of the sample of extracted product obtained at 10% ethanol, 30 MPa, 50 °C and 20 kg h^{-1} is shown in Fig 3. In Fig 3, the peak eluting at 2.42 min was ascribed to all-*trans*-piceid and the peak at 4.22 min was all-*trans*-resveratrol when compared with the standard sample.

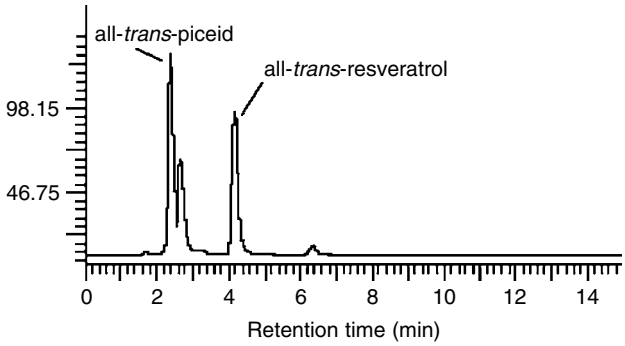

Figure 3. HLPC chromatogram of the supercritical CO_2 extracts of *Polygonum cuspidatum* obtained at 30 MPa, $T = 50$ °C, $vCO_2 = 20$ kg h^{-1}, ethanol = 100 ml l^{-1}.

Table 2. The yields of resveratrol and piceid obtained by SFE and organic solvent extraction (OSE)

	SFE[a]		OSE[b]	
Item	Resveratrol	Piceid	Resveratrol	Piceid
Yields (% (SD), g kg^{-1})	7.1 (0.4)	16.0 (1.2)	7.7 (0.5)	17.3 (1.4)

[a] SFE: 100 ml l^{-1} of ethanol, 30 MPa, 50 °C, 20 kg h^{-1};
[b] OSE: ethanol (950 ml l^{-1}), 75 °C, 2 h.

Comparison of SFE and organic solvent extraction

Table 2 compares the extraction efficiency of SFE with organic solvent extraction (OSE). As could be seen, SFE gained yields of resveratrol (7.1 g kg^{-1}) and piceid (16.0 g kg^{-1}), which was comparable with OSE. The result was consistent with previous reports in the literature[17,18] that the reverstrol and piceid contents in the roots of *P cuspidatum* were 1–10.7 g kg^{-1} and 12 ~ 25 g kg^{-1} respectively.

Because of increasingly stringent environmental regulations, SFE has gained wide acceptance in recent years as an alternative to conventional solvent extraction for separation of organic compounds. Our result indicated that using supercritical CO_2 plus ethanol modifier was a feasible way extract of resveratrol and piceid from *P cuspidatum*. The optimal conditions for extraction of resveratrol and piceid were: 100 ml l^{-1} of ethanol as modifier, 30 MPa, 50 °C, 20 kg h^{-1} of CO_2.

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